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## **Disruption of multisystem responses to stress in type 2 diabetes: investigating the dynamics of allostatic load**

Steptoe, Andrew ; Hackett, Ruth A ; Lazzarino, Antonio I ; Bostock, Sophie ; La Marca, Roberto ;  
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**Abstract:** Psychological stress-related processes are thought to contribute to the development and progression of type 2 diabetes, but the biological mechanisms involved are poorly understood. Here, we tested the notion that people with type 2 diabetes experience chronic allostatic load, manifest as dynamic disturbances in reactivity to and recovery from stress across multiple (cardiovascular, neuroendocrine, inflammatory, metabolic) biological systems, coupled with heightened experience of chronic life stress. We carried out an experimental comparison of 140 men and women aged 50-75 y with type 2 diabetes and 280 nondiabetic individuals matched on age, sex, and income. We monitored blood pressure (BP) and heart rate, salivary cortisol, plasma interleukin (IL)-6, and total cholesterol in response to standardized mental stress, and assessed salivary cortisol over the day. People with type 2 diabetes showed impaired poststress recovery in systolic and diastolic BP, heart rate and cholesterol, and blunted stress reactivity in systolic BP, cortisol, cholesterol, and IL-6. Cortisol and IL-6 concentrations were elevated, and cortisol measured over the day was higher in the type 2 diabetes group. Diabetic persons reported greater depressive and hostile symptoms and greater stress experience than did healthy controls. Type 2 diabetes is characterized by disruption of stress-related processes across multiple biological systems and increased exposure to life stress. Chronic allostatic load provides a unifying perspective with implications for etiology and patient management.

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# Disruption of multisystem responses to stress in type 2 diabetes: Investigating the dynamics of allostatic load

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Psychological stress-related processes are thought to contribute to the development and progression of type 2 diabetes, but the biological mechanisms involved are poorly understood. Here, we tested the notion that people with type 2 diabetes experience chronic allostatic load, manifest as dynamic disturbances in reactivity to and recovery from stress across multiple (cardiovascular, neuroendocrine, inflammatory, metabolic) biological systems, coupled with heightened experience of chronic life stress. We carried out an experimental comparison of 140 men and women aged 50–75 y with type 2 diabetes and 280 nondiabetic individuals matched on age, sex, and income. We monitored blood pressure (BP) and heart rate, salivary cortisol, plasma interleukin (IL)-6, and total cholesterol in response to standardized mental stress, and assessed salivary cortisol over the day. People with type 2 diabetes showed impaired poststress recovery in systolic and diastolic BP, heart rate and cholesterol, and blunted stress reactivity in systolic BP, cortisol, cholesterol, and IL-6. Cortisol and IL-6 concentrations were elevated, and cortisol measured over the day was higher in the type 2 diabetes group. Diabetic persons reported greater depressive and hostile symptoms and greater stress experience than did healthy controls. Type 2 diabetes is characterized by disruption of stress-related processes across multiple biological systems and increased exposure to life stress. Chronic allostatic load provides a unifying perspective with implications for etiology and patient management.

inflammation | neuroendocrine | depression | cardiovascular | metabolic

Type 2 diabetes has a heterogeneous pathophysiology in which  $\beta$ -cell dysfunction and insulin resistance play pivotal roles (1). Stress-related factors may contribute to risk of type 2 diabetes through their impact on inflammatory, metabolic, cardiovascular, and neuroendocrine regulation (2). Socioeconomic adversity over the life course predicts type 2 diabetes in later life (3), whereas stress at work and more general indicators of perceived stress are associated with future diabetes (4, 5). There appears to be a bidirectional relationship between type 2 diabetes and depressive symptoms (6), and people with type 2 diabetes may report greater social isolation and more limited social support (7).

These diverse associations between stress-related processes and diabetes are only partly accounted for by lifestyle factors such as physical inactivity, alcohol consumption, or adiposity, suggesting that direct psychobiological pathways may be involved. A helpful concept in this regard is allostatic load. Allostasis refers to the dynamic process of adaptation to environmental challenges through adjustments in multiple biological systems, including the hypothalamic–pituitary–adrenocortical (HPA) axis, autonomic nervous system, and metabolic and immune systems (8). Allostasis is essential for maintaining homeostasis, but repeated or sustained stimulation leads to allostatic load, the wear-and-tear that results from dysregulation of mediating processes. Allostatic load is frequently quantified by measuring a range of biomarkers (e.g., blood pressure, cortisol, catecholamines, glycated hemoglobin, waist circumference, cholesterol, inflammatory markers), and allocating individuals scores based on the number of variables on which values are elevated compared with the sample distribution (9, 10). Studies relating allostatic load measures with type 2 diabetes

have been inconsistent to date (11, 12). However, another aspect of allostasis is that it is manifest in modifications of dynamic responses to challenge, not only in basal measures. Adaptive biological responses to stress involve brisk increases in activation (stress reactivity) as the person mobilizes for vigorous activity, followed by prompt recovery back to baseline levels. McEwen (8) has noted that high allostatic load disrupts these dynamic biological responses, resulting in changes in the morphology of responses, notably impaired poststress recovery and inadequate biological responses (blunted stress reactivity).

Accordingly, we tested the notion that type 2 diabetes is characterized by high dynamic allostatic load. We hypothesized that in response to standardized mental stress, people with type 2 diabetes would show impaired poststress recovery and blunted stress reactivity in blood pressure (BP), heart rate, cortisol, and serum cholesterol, together with greater inflammation indexed by interleukin (IL)-6. We monitored cortisol output in everyday life by obtaining multiple saliva samples over the day to test whether people with type 2 diabetes have higher cortisol output in naturalistic settings. We also measured relevant psychological variables, conjecturing that people with diabetes would report more emotional distress, greater stress in their lives, and reduced psychosocial resources compared with nondiabetic individuals.

## Results

We compared 140 men and women aged 50–75 y with type 2 diabetes with 280 nondiabetic individuals matched by age, sex, and income category. The diabetic participants had better education and were less likely to be married than were controls (Table 1). As expected, glycated hemoglobin (HbA1c), body mass index (BMI), and waist circumference were greater in the

## Significance

Our observations provide evidence to link epidemiological studies implicating stress-related processes with biological dysfunction in type 2 diabetes. The patterns of cardiovascular, inflammatory, neuroendocrine, and cholesterol responses exemplify the disturbances of reactivity and recovery predicted by the allostatic load model, including prolonged responses to stress due to delayed shutdown of physiological reactivity, and inadequate (blunted) responses resulting in compensatory hyperactivity in other mediating pathways. Chronic allostatic load may be a mechanism through which stress exposures contribute to diabetes risk, while also being implicated in the adverse health consequences of diabetes such as coronary heart disease and cognitive decline.

Author contributions: A.S., R.L.M., L.A.C., and M.H. designed research; R.A.H., A.I.L., S.B., and L.A.C. performed research; R.L.M. contributed new reagents/analytic tools; A.S. and R.A.H. analyzed data; and A.S. and R.A.H. wrote the paper.

The authors declare no conflict of interest.

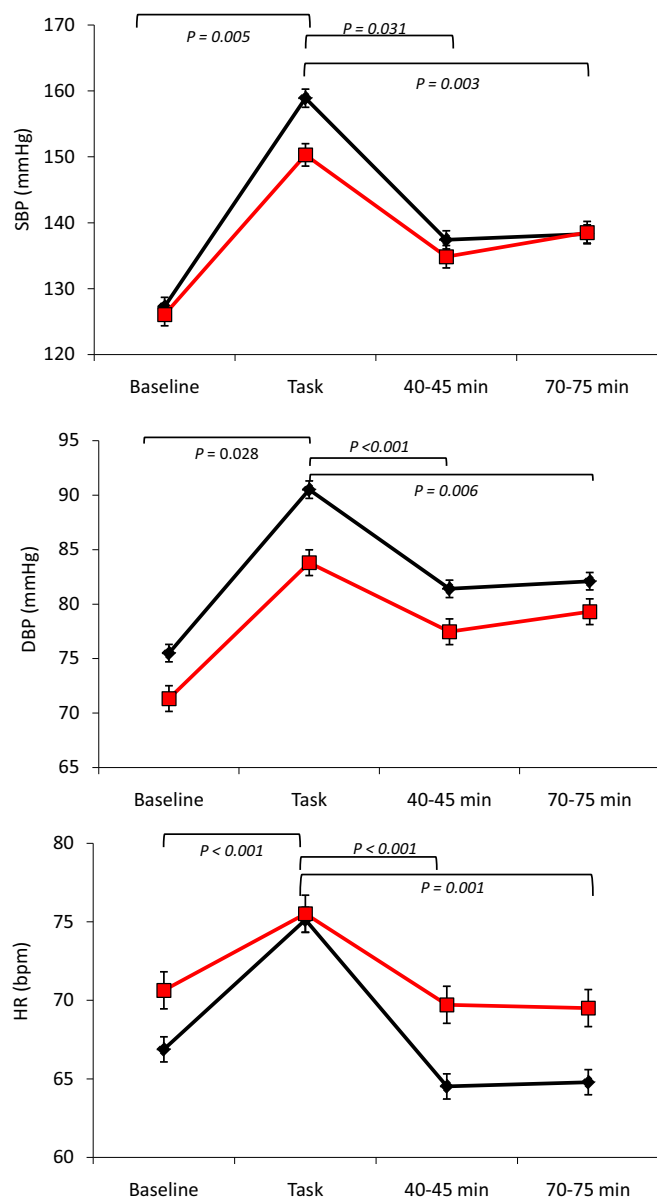
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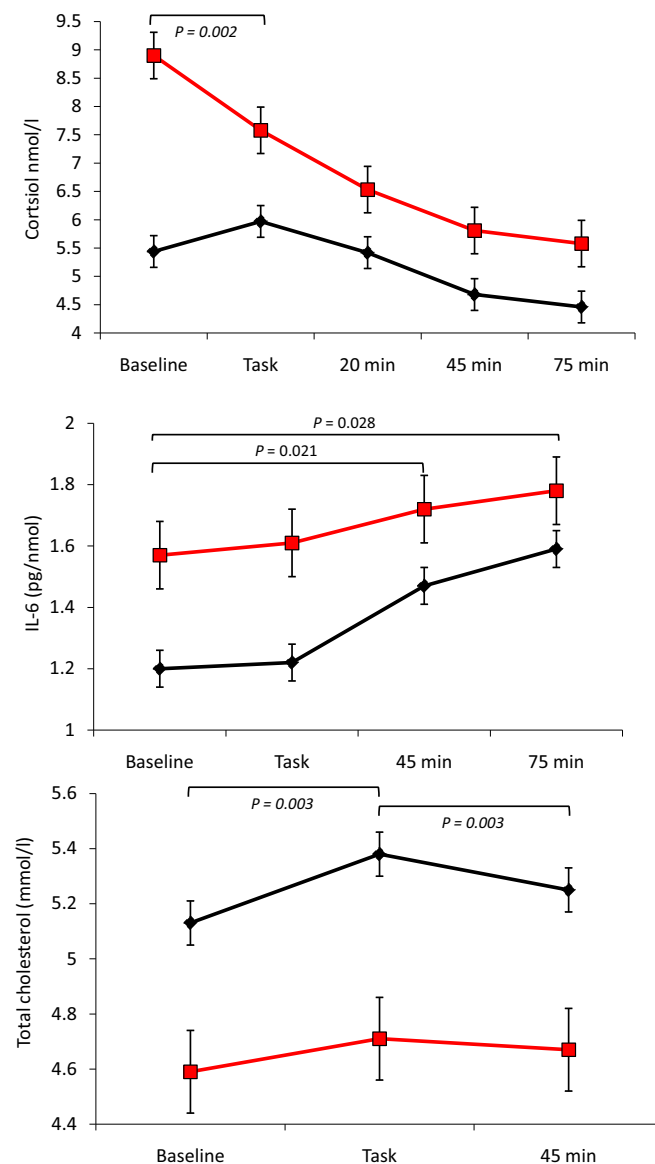
**Fig. 1.** Mean systolic BP (Upper), diastolic BP (Center), and heart rate (Lower) during baseline, task trials, and 40–45 min and 70–75 min posttasks in the diabetes (red line) and control (black line) groups. BP and heart rate data are adjusted for education, marital status, BMI, smoking status, beta-blocker use, and time of testing. *P* values indicate group differences in stress reactivity (baseline–task difference) and poststress recovery (task–posttask differences) as detailed in Table S1. Error bars are SEMs.

**Intercorrelation Between Responses in Different Systems.** There were consistent associations between responses in the different systems monitored in this study. Thus, cardiovascular (BP and heart rate) reactions to tasks were negatively correlated with cortisol output over the day ( $P < 0.05$ ) and with baseline IL-6 concentration ( $P < 0.01$ ), while being positively related to cortisol ( $P < 0.01$ ) and cholesterol ( $P < 0.001$ ) responses to mental stress. Measures of poststress recovery showed similar patterns. Interrelationships between biological responses and psychological characteristics were also present. For example, systolic BP reactivity was negatively correlated with depressive symptoms, financial strain, and hostility, and positively associated with social cohesion, social support, control over life, and optimism, after adjustment for group status (all  $P < 0.05$ ). Baseline IL-6 concentration was positively related to depressive symptoms and financial

strain ( $P < 0.05$ ), and negatively with social support ( $P = 0.024$ ). Cortisol responses to acute stress were positively related to social cohesion ( $P < 0.05$ ) and social support ( $P = 0.009$ ), whereas individuals with greater cortisol output over the day were low in optimism ( $P < 0.001$ ).

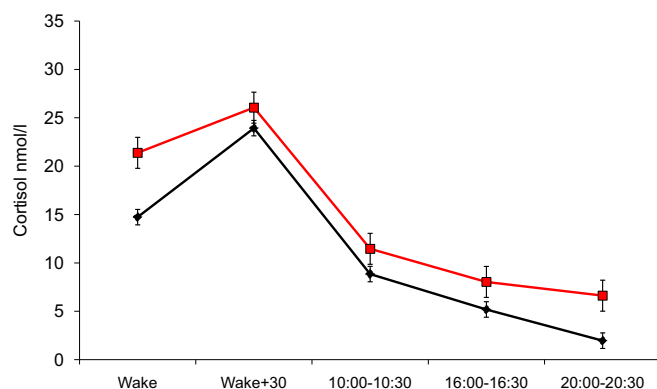
## Discussion

This study explored the hypothesis that people with type 2 diabetes experience chronic allostatic load, manifest in alterations in dynamic physiological responses to standardized mental stress,



**Fig. 2.** Mean salivary cortisol (Upper; geometric means), plasma IL-6 (Center; geometric means), and total cholesterol (Lower) in the diabetes (red line) and control (black line) groups. Cortisol sampled at baseline, immediately after tasks, and 20, 45, and 75 min later. Values are adjusted for education, marital status, BMI, smoking status, use of statins, and time of testing. The *P* value indicates group differences in stress reactivity. IL-6 was adjusted for education, marital status, BMI, smoking, and time of testing; *P* values indicate group differences in stress reactivity. Total cholesterol was adjusted for education, marital status, BMI, smoking, aspirin, use of statins, and time of testing. *P* values indicate group differences in stress reactivity (baseline–task difference) and poststress recovery (task–posttask difference). Error bars are SEM.





**Fig. 3.** Mean salivary cortisol sampled on waking, 30 min after waking, and in the morning (1000–1030), afternoon (1600–1630), and evening (2000–2030) in the diabetes (red line) and control (black line) groups. Values are adjusted for education, marital status, BMI, smoking status, oral diabetic medication, and beta-blocker use. Error bars are SEM.

higher cortisol output over the day, and greater psychological distress and experience of chronic life stress, in comparison with age, sex, and income matched controls. We found that poststress recovery was attenuated in the diabetes group in systolic and diastolic BP, heart rate, and total cholesterol, together with blunted stress reactivity in BP, heart rate, cortisol, and cholesterol concentration. These effects were independent of covariates including medication and were evident in both men and women. The acute increases in cholesterol concentration are likely to be due in part to reductions in blood volume following stress, leading to greater hemoconcentration. Plasma IL-6 concentration was higher in people with type 2 diabetes, so that although the increases following mental stress were smaller than those of controls, absolute levels remained higher. The type 2 diabetes group had higher baseline cortisol than controls in the laboratory, together with heightened cortisol output throughout the day. We also found that people with type 2 diabetes had more depressive and hostile symptoms, and reported greater chronic stress in terms of financial and neighborhood strain.

The multisystem measures of allostatic load described in the literature typically involve measures taken under resting conditions and include several components of the metabolic syndrome such as elevated BP, triglycerides, and waist circumference (13), so it is surprising that the concept has not been successfully applied to type 2 diabetes. One study of middle-aged Puerto Ricans living in Boston showed that greater allostatic load was associated with increased risk of diabetes and other chronic conditions (12), but another investigation focusing specifically on diabetes failed to find that the components of allostatic load clustered reliably in people with diabetes (11).

Our results suggest that attention to the dynamic aspects of allostatic load may be fruitful. Our systematic review of published literature found no previous studies that have examined the dynamic responses to mental stress across multiple biological systems in type 2 diabetes. One small study showed that systolic BP responses to a mental arithmetic task were greater in diabetes than healthy groups, but it involved an inappropriate comparison group and did not use continuous measures of BP (14). Three studies have compared diurnal salivary cortisol profiles in people with and without type 2 diabetes: Two reported smaller cortisol responses to waking in diabetic participants (15, 16) and one a flatter slope over the day (17), but differences in cortisol output over the day have been inconsistent. We observed striking elevations in cortisol at baseline and in everyday life in people with type 2 diabetes, and elevated cortisol has been implicated in the development of the metabolic syndrome (18). Heightened cortisol output is also thought to be involved in the development of obesity, but we controlled statistically for BMI in these analyses. Blunted cortisol

stress reactivity may have a permissive effect on IL-6 in type 2 diabetes. The high IL-6 concentration is likely related to the role of inflammation in type 2 diabetes, promoting insulin resistance and dyslipidemia by inhibiting enzymes involved in fatty acid oxidation, down-regulating the expression of genes involved in insulin-stimulated glucose transport and lipid uptake in adipocytes (19, 20).

Apart from heightened dynamic allostatic load, one alternative explanation for our results is that people with diabetes were less stressed by the behavioral challenges, so they produced smaller biological responses; however, the subjective ratings indicate that the diabetes and control groups were stressed to the same extent by the tasks. A second possibility is that effects were confounded by the multiple medications used to control diabetes. These medications may have contributed to the low baseline levels of diastolic BP and total cholesterol observed in the diabetes group (Figs. 1 and 2), but we took account statistically of medications that had associations with biological stress responses. Third, it is conceivable that the differences in BP and heart rate were early manifestations of neuropathy in people with diabetes, although none had clinical signs of cardiac autonomic neuropathy (21).

Our observation that depressive symptoms were elevated in people with type 2 diabetes replicates previous research (6) and is important in light of the evidence that depression in diabetic populations is associated with accelerated cognitive decline (22) and increased mortality risk (23). It is interesting that all of the psychosocial measures except for caregiving indicated greater stress among people with diabetes than controls. The result is a profile of psychosocial adversity in the type 2 diabetes group likely to promote heightened allostatic load.

Measures of allostatic load have been criticized for bringing together an arbitrary set of biomarkers, assuming that extreme values load on an underlying unitary construct. However, recent factor analytic studies indicate that a single common factor underlies variation across autonomic, neuroendocrine, inflammatory, and metabolic processes (13, 24). The intercorrelations between responses in the different biological systems, and between biological responses and psychosocial factors, provide further support for the value of this approach.

The study was cross-sectional, so no causal conclusions can be drawn. It is possible that heightened allostatic load precedes the development of type 2 diabetes and is a mechanism through which psychosocial factors contribute to diabetes risk. There are direct effects of inflammation on  $\beta$  cells (1), and heightened inflammation predicts diabetes onset (25). Autonomic and glucocorticoid regulation are also implicated in central fat deposition and the metabolic syndrome (26, 27), so they may contribute to the development of diabetes. Studies of heightened dynamic allostatic load in people with insulin resistance but no diabetes would throw light of this pathway. The alternative is that allostatic load is a manifestation of diabetes that is secondary to the abnormalities of glucose metabolism. The cardiovascular, neuroendocrine, and inflammatory responses we observed may be significant for the broader health consequences of diabetes. Disturbances of cortisol regulation are apparent in coronary heart disease and depression (28, 29), and it has been argued that glucocorticoids may contribute to the development of cognitive impairment in people with diabetes (30). Chronic systemic inflammation is also involved in cardiovascular disease, dementia, and depression (31, 32). Our results are consistent with the possibility that some of the comorbidities of type 2 diabetes arise from the disruption of the multiple systems involved in allostatic load rather than being direct consequences of impaired glucose regulation.

The dynamic aspects of multisystem dysregulation in allostatic load have not been studied prospectively in relation to long-term health outcomes. However, other measures of allostatic load predict future mortality and functional decline in older people (9). Individual components such as reduced cardiovascular poststress recovery, blunted stress reactivity, and increased IL-6 during acute stress are also associated with future adverse health outcomes (33).

**Table 2. Psychosocial factors in the diabetes and healthy control groups**

	Diabetes*	Healthy control*	Odds of diabetes <sup>†</sup> (95% C.I.)	P
Depression symptoms	11.86 ± 8.9	6.47 ± 6.6	1.16 (1.11–1.22)	0.001
Hostility	3.78 ± 2.8	2.72 ± 2.4	1.20 (1.08–1.37)	0.001
Financial strain	84 (71.2%)	122 (43.7%)	1.38 (1.24–1.53)	0.001
Social cohesion	9.83 ± 5.5	11.62 ± 4.6	0.91 (0.86–0.96)	0.001
Caregiver	24 (17.1%)	37 (13.2%)	1.37 (0.74–2.56)	0.32
Sense of control	3.29 ± 1.6	3.92 ± 1.4	0.77 (0.65–0.91)	0.002
Optimism	14.4 ± 4.3	15.5 ± 3.8	0.91 (0.86–0.96)	0.002
Social support	25.7 ± 7.1	26.3 ± 5.4	0.99 (0.95–1.04)	0.72

\*Mean ± SD, or n (%).

<sup>†</sup>Odds of being in the diabetes group per unit change in the independent variable, adjusted for education and marital status.

In addition to the cross-sectional design, other limitations of this study are that the diabetes group was more ethnically diverse than the control group, and that the assessment of stress responses was carried out over a single session. Further, we did not measure glucose and insulin responses across the mental stress session. Nonetheless, our observations provide fresh evidence to link epidemiological studies implicating stress-related processes with biological dysfunction in type 2 diabetes. The patterns of cardiovascular and cholesterol responses exemplify the disturbances of reactivity and recovery noted in McEwen's model of allostatic load (8). It has been posited that high allostatic load leads to prolonged responses due to delayed shutdown of physiological reactivity, so poststress recovery is impeded. Blunted reactivity may also occur, resulting in compensatory hyperactivity in other mediating pathways. The allostatic load concept synthesizes diverse findings concerning stress-related dysregulation across cardiovascular, neuroendocrine, metabolic, and inflammatory systems. Our findings highlight the importance of moving beyond glucose regulation to address a range of disturbances across multiple systems. Although individual pathways can be targeted by pharmacotherapy, the allostatic approach implies that systems interact in a dynamic fashion. Interventions that affect both brain and body are likely to be particularly beneficial. Two promising candidates are physical activity and social integration, both of which are implicated in diabetes (34). Development of the allostatic approach to type 2 diabetes may open new avenues for pharmacological and social-behavioral approaches to management and prevention.

## Materials and Methods

**Participants.** One hundred and forty individuals with type 2 diabetes were recruited from diabetic outpatient and primary care clinics in the London area with an invitation to take part in an investigation of how people with type 2 diabetes respond to stress. We limited enrollment to patients without a history or previous diagnosis of coronary heart disease (CHD), inflammatory diseases, allergies or mood disorders, and no evidence of autonomic neuropathy. Respondents included 88 men and 52 women aged 50–75 y.

The healthy controls in this analysis came from the Heart Scan Study, a subsample of the Whitehall II epidemiological cohort recruited between 2006 and 2008 to investigate socioeconomic and psychosocial factors, physiological stress responsivity, and subclinical coronary artery disease (35). Volunteers were invited to take part in a study of how people respond to stress, and a sample of 543 men and women of white European origin with no history or objective signs of CHD, no previous diagnosis or treatment for hypertension, diabetes, inflammatory diseases, or allergies was recruited. The absence of diabetes was confirmed by low HbA1c levels ( $\leq 6.5\%$ , or 48 mmol/mol) and negative oral glucose tolerance tests over the previous 20 y. We matched every person with diabetes as closely as possible by age, sex, and income category with two healthy controls, so the 140 diabetic respondents were compared with 280 nondiabetic individuals. All participants gave full informed consent, and ethical approval was obtained from the National Research Ethics Service.

**Mental Stress Testing.** We tested participants individually either in the morning or afternoon. BP and heart rate were monitored continuously by

using a Finometer, and a venous cannula was inserted for blood collection. Baseline blood and saliva samples were collected, and subjective stress ratings were obtained on a seven-point scale from 1 = no stress to 7 = maximum stress. We then administered two 5-min behavioral tasks in random order. These were a computerized version of the Stroop color-word interference task and a mirror drawing task as described (35). These tasks were originally designed as tests of cognitive function, but were adapted to provide moderately stressful behavioral challenges. Subjective stress, blood, and saliva samples were taken immediately after tasks. The posttask recovery period lasted 75 min, with further saliva samples at 20, 45, and 75 min and additional blood samples and stress ratings at 45 and 75 min. Blood samples were centrifuged immediately at  $400 \times g$  for 10 min at room temperature and plasma was separated and stored at  $-80^\circ\text{C}$  until analysis.

**Cortisol Output over the Day.** Participants collected five saliva samples over a single day by using Salivettes (Sarstedt), at waking, 30 min later, and then within three 30-min time windows in the morning (1000–1030), afternoon (1600–1630) and evening (2000–2030). They were told not to eat, drink tea or coffee, or smoke in the 30 min before each sample was collected. Violations of the protocol and sample timing were recorded in a log. Satisfactory data were obtained from 119 diabetic and 236 nondiabetic participants.

**Biological Measures.** Plasma IL-6 was assayed by using a Quantikine high sensitivity two-site ELISA (R&D Systems). The sensitivity of the assay ranged from 0.016 to 0.110 pg/mL, and the intraassay and interassay coefficient of variations was 7.3% and 7.7% respectively. Cortisol was assessed from saliva samples by using a time resolved immunoassay with fluorescence detection at the University of Dresden, Germany. Total cholesterol was measured in a centrifugal analyzer by enzymatic colorimetric methods. Plasma IL-6 was assayed from all four blood samples, HbA1c from the baseline sample alone, and total cholesterol from the baseline, task, and 45 min posttask samples.

**Psychological Measures.** We measured two stress-related psychological characteristics, depressive symptoms and hostility, using the Center for Epidemiologic Studies Depression Scale and the 10-item Cook–Medley cynical hostility scale, respectively. Stress exposure was assessed in terms of financial strain and neighborhood social cohesion with a measure of collective efficacy (36). We also noted whether the participant was a caregiver for an elderly or disabled relative or friend. Participants rated their sense of control over their lives in general by using a six-point scale from 1 = very low to 6 = very high, whereas social support and optimism were assessed with standardized measures (37, 38). The Cronbach  $\alpha$  was satisfactory for all psychological measures ( $>0.75$ ).

**Other Measures.** Participants reported household income in bands that were subsequently reduced to lower ( $<£40,000$ ) and higher ( $\geq£40,000$ ) income categories. Education was categorized into less than high school, high school or equivalent, and college or higher. Medication in the diabetes group was categorized into oral diabetic medication (metformin), insulin and other injected diabetic medication, aspirin, beta blockers, other hypertensive medication (ACE inhibitors, calcium channel blockers), and statins.

**Statistical Analysis.** We computed mean systolic BP, diastolic BP, and heart rate for five periods: baseline (the last 5 min of the 30-min baseline period), the two task trials combined, and poststress recovery minutes 40–45 and 70–75. We conducted repeated measures analysis of variance to assess patterns of change across the session, with group and sex as between-person factors and trial as the within-person factor. Because this study was a matched case-control

design, analysis of variance and general estimating equation models were not appropriate for comparisons between groups. Instead, we analyzed differences between groups in stress reactivity and stress recovery by using conditional logistic regression, which takes account of the matched case-control design (39). We computed difference scores between tasks and baseline (for stress reactivity) and differences between tasks and recovery measures (for recovery analyses), and entered these along with covariates into regressions on group (diabetes or control) membership. Results are presented as adjusted odds of being in the diabetes as opposed to the control group per unit change in the predictor variable, with 95% confidence intervals (95% CI). A value  $>1$  indicates that larger values of the predictor are associated with increased odds of being in the diabetes group, while values  $<1$  indicate that larger values are associated with reduced odds of being in the diabetes group. Stress responses in IL-6 are delayed in comparison with other measures, so we assessed reactivity as differences between values recorded at 45 min and 75 min compared with baseline. Poststress recovery in cardiovascular measures, cortisol, and cholesterol was measured by using difference scores between task and recovery period means. We log transformed cortisol and IL-6 before analysis because of positive skewness in the distribution, and geometric means are presented in *Results*. Cortisol output over the day was quantified as area under the curve (40). There were 28 (20%) ethnic minority participants in the

diabetes group, but adding ethnicity as a factor to the analyses did not alter the pattern of results, so it is not included in the models described here.

All analyses of physiological data were adjusted for BMI and smoking status, because these variables are known to influence stress responses. Time of day of stress testing was included as a covariate, because profiles of response to stress may vary across the day. We also adjusted for education and marital status because of differences between groups, because these factors might affect stress responsivity. We selected medication covariates by testing for associations between medication status and stress responses within the diabetes group. Thus, BP and HR analyses were additionally adjusted for use of beta blockers in addition to education, marital status, BMI, and smoking; cortisol analyses for statins; and cholesterol responses for use of statins and aspirin. Psychological differences between groups were analyzed by using conditional logistic regression adjusted for education and marital status. We also explored interrelationships between cardiovascular and other biological responses, and between biological responses and psychological factors, by computing product-moment correlations.

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